

**What is claimed is:**

1. A method for assaying homocysteine (Hcy), S-adenosylhomocysteine (SAH) or adenosine in a sample, which method comprises:

5        a) contacting a sample containing or suspected of containing Hcy, SAH or adenosine with a mutant SAH hydrolase, wherein said mutant SAH hydrolase has binding affinity for Hcy, SAH or adenosine but has attenuated catalytic activity, and said binding affinity and/or said attenuated catalytic activity of said SAH hydrolase is caused by a mutation in said mutant SAH hydrolase's catalytic site, its binding site for NAD<sup>+</sup>, NADH, Hcy, SAH or adenosine, or a

10 combination thereof; and

      b) detecting binding between Hcy, SAH or adenosine with said mutant SAH hydrolase,

      whereby the presence or amount of Hcy, SAH or adenosine in said sample is assessed.

15        2. The method of claim 1, wherein the mutant SAH hydrolase:

      a) has a mutation in an amino acid residue that is directly involved in the SAH hydrolase's catalytic activity, its binding with NAD<sup>+</sup>, NADH, Hcy, SAH or adenosine; or  
      b) has a mutation in an amino acid residue that is adjacent to an amino acid residue that is directly involved in the SAH hydrolase's catalytic activity, its binding with NAD<sup>+</sup>, NADH, Hcy, SAH or adenosine.

20        3. The method of claim 1, wherein the mutant SAH hydrolase has enhanced binding affinity for Hcy, SAH or adenosine than a wild type SAH hydrolase from which said mutant SAH hydrolase is derived.

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      4. The method of claim 3, wherein the mutant SAH hydrolase has at least 50 fold higher binding affinity for Hcy, SAH or adenosine than a wild type SAH hydrolase from which said mutant SAH hydrolase is derived.

5. The method of claim 1, wherein the mutant SAH hydrolase is derived from a mammalian SAH hydrolase.

6. The method of claim 1, wherein the mutant SAH hydrolase is derived from a human SAH hydrolase.

7. The method of claim 1, wherein the mutant SAH hydrolase comprises the amino acid sequence set forth in SEQ ID NO:1 and comprises a mutation selected from the group consisting of R38E, C53S, L54G, T57G, T57S, E59D, N80G, S83G, Y100T, K121A, D131E, D134E, E155G, T157G, T158Y, T159Y, N181D, N181A, D190A, N191A, L214A, Y221S, K226A, F235S, I240L, N248A, D263G, G269D, R285D, D292G, H301T, K309R, K322G, R329A, L347F, L347Y, L347I, M351A, H353R, S361G, F362S, Y379S, L386A, K388G, H398A, K401R, K401D, T407S, L409G, S420T, P424A, F425S, P427A, D428G, H429A, Y430T, R431K, R431G, Y432S, Y432A, Y432F, and a combination thereof.

15 8. The method of claim 1, wherein prior to the contact between the sample and the mutant SAH hydrolase, oxidized or conjugated Hcy in the sample is converted into reduced Hcy.

20 9. The method of claim 1, wherein prior to the contact between the sample and the mutant SAH hydrolase, the Hcy in the sample is converted into SAH.

10. The method of claim 9, wherein the Hcy in the sample is converted into SAH by a wild-type SAH hydrolase and access adenosine.

25 11. The method of claim 10, wherein the access adenosine in the sample is removed by adenosine deaminase while the wild-type SAH hydrolase is inhibited.

12. The method of claim 11, wherein the wild-type SAH hydrolase inhibitor is neplanocin A or aristomycin.

13. The method of claim 8, further comprising a step of removing the reducing agent used to convert oxidized or conjugated Hcy into reduced Hcy prior to or concurrently with contacting the sample with the mutant SAH hydrolase.

5 14. The method of claim 13, wherein the reducing agent is removed by chromatography.

15. The method of claim 14, wherein the chromatography is a batch chromatography.

10 16. The method of claim 1, wherein an indicator dye is used and the indicator dye is removed by chromatography prior to or concurrently with contacting the sample with the mutant SAH hydrolase.

15 17. The method of claim 16, wherein the chromatography is a batch chromatography.

18. The method of claim 1, wherein the SAH is contacted with the mutant SAH hydrolase in the presence of a labeled SAH or a derivative or an analogue thereof, thereby the amount of the mutant SAH hydrolase bound to the labeled SAH inversely relates to the amount of SAH in the sample.

20 19. The method of claim 18, wherein the labeled SAH or a derivative or an analogue thereof is fluorescently, enzymatically or proteinaceously labeled.

25 20. The method of claim 19, wherein the fluorescently labeled SAH is fluorescin-SAH conjugate or Rocamin-SAH conjugate, said fluorescin or Rocamin being linked to said SAH or a derivative or an analogue thereof by a linker of 1-15 carbon atom length.

30 21. The method of claim 19, where the enzymatically labeled SAH derivative is Glucose-6-phosphate dehydrogenase (G-6-PDH-SAH) conjugate, alkaline phosphatase-SAH conjugate, or malate dehydrolase-SAH conjugate, said G-6-PDH, alkaline phosphatase or malate

dehydrolase being linked to said SAH or a derivative or an analogue thereof by a linker of 1-15 carbon atom length.

22. The method of claim 19, wherein the proteinaceous labeled SAH derivative is

5 bovine albumin-SAH conjugate, said bovine albumin being linked to said SAH or a derivative or an analogue thereof by a linker of 1-15 carbon atom length.

23. The method of claim 1, wherein the mutant SAH hydrolase is a labeled mutant SAH hydrolase.

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24. The method of claim 23, wherein the labeled mutant SAH is a fluorescently, enzymatically, biotin or streptavidin labeled mutant SAH hydrolase.

15 25. The method of claim 24, wherein the biotin labeled mutant SAH hydrolase is detected by a streptavidin labeled enzyme.

26. The method of claim 25, wherein the streptavidin labeled enzyme is a streptavidin labeled horse radish phosphatase (HRP).

20 27. The method of claim 22, wherein the bovine albumin-SAH conjugate is immobilized.

28. The method of claim 19, wherein the fluorescently labeled SAH or a derivative or an analogue thereof is directly contacted by the mutant SAH hydrolase, and the resulting change of 25 fluorescent polarization is measured for assessing Hcy, SAH or adenosine.

29. The method of claim 19, wherein the enzymatically labeled SAH or a derivative or an analogue thereof is directly contacted by the mutant SAH hydrolase, and the resulting change of enzyme activity is measured for assessing Hcy, SAH or adenosine.

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30. The method of claim 1, wherein the mutant SAH hydrolase is immobilized.

31. The method of claim 1, wherein the sample is a body fluid or a biological tissue.

5 32. The method of claim 31, wherein the body fluid is selected from the group consisting of urine, blood, plasma, serum, saliva, semen, stool, sputum, cerebral spinal fluid, tears, mucus and amniotic fluid.

10 33. The method of claim 31, wherein the body fluid is blood.

34. The method of claim 33, wherein the blood sample is further separated into a plasma or serum fraction.

15 35. The method of claim 1, further comprising detecting cholesterol and/or folic acid in the sample.

36. A combination, which combination comprises:

20 a) a mutant SAH hydrolase that has binding affinity for Hcy, SAH or adenosine but has attenuated catalytic activity, wherein said binding affinity and/or said attenuated catalytic activity of said SAH hydrolase is caused by a mutation in said mutant SAH hydrolase's catalytic site, its binding site for NAD<sup>+</sup>, NADH, Hcy, SAH or adenosine, or a combination thereof; and  
b) reagents for detecting binding between Hcy, SAH or adenosine and said SAH hydrolase.

25 37. The combination of claim 36, further comprising a reagent for detecting cholesterol and/or folic acid.

38. A kit, which kit comprises the combination of claim 36.

39. The kit of claim 38, further comprising instructions for assaying Hcy, SAH or adenosine in a sample.

40. An article of manufacture, which article of manufacture comprises:

- 5 a) packaging material;
- b) a mutant SAH hydrolase that has binding affinity for Hcy, SAH or adenosine but has attenuated catalytic activity, wherein said binding affinity and/or said attenuated catalytic activity of said SAH hydrolase is caused by a mutation in said mutant SAH hydrolase's catalytic site, its binding site for NAD<sup>+</sup>, NADH, Hcy, SAH or adenosine, or a combination thereof; and
- 10 c) a label indicating that the mutant SAH hydrolase and the means for use in assaying Hcy in a sample.

15 41. An isolated nucleic acid fragment, which isolated nucleic acid fragment comprises a sequence of nucleotides encoding a mutant SAH hydrolase, wherein said mutant SAH hydrolase comprises the amino acid sequence set forth in SEQ ID NO:1 and comprises a mutation selected from the group consisting of R38E, C53S, L54G, T57G, T57S, E59D, N80G, S83G, Y100T, K121A, D131E, D134E, E155G, T157G, T158Y, T159Y, N181D, N181A, D190A, N191A, L214A, Y221S, K226A, F235S, I240L, N248A, D263G, G269D, R285D, D292G, H301T, K309R, K322G, R329A, L347F, L347Y, L347I, M351A, H353R, S361G, F362S, Y379S, L386A, K388G, 20 H398A, K401R, K401D, T407S, L409G, S420T, P424A, F425S, P427A, D428G, H429A, Y430T, R431K, R431G, Y432S, Y432A, Y432F, and a combination thereof.

42. The isolated nucleic acid fragment of claim 41, wherein the nucleic acid is DNA.

25 43. The isolated nucleic acid fragment of claim 41, wherein the nucleic acid is RNA.

44. A plasmid, which plasmid comprises the nucleic acid fragment of claim 41.

45. A cell, which cell comprises the plasmid of claim 44.

46. The cell of claim 45 selected from the group consisting of a bacterial cell, a yeast cell, a fungal cell, a plant cell, an insect cell and an animal cell.

47. A method for producing a mutant SAH hydrolase, which method comprises  
5 growing the cell of claim 45 under conditions whereby the mutant SAH hydrolase is expressed by the cell, and recovering the expressed mutant SAH hydrolase.

48. A substantially purified mutant SAH hydrolase, wherein said mutant SAH hydrolase comprises the amino acid sequence set forth in SEQ ID NO:1 and comprises a mutation  
10 selected from the group consisting of R38E, C53S, L54G, T57G, T57S, E59D, N80G, S83G, Y100T, K121A, D131E, D134E, E155G, T157G, T158Y, T159Y, N181D, N181A, D190A, N191A, L214A, Y221S, K226A, F235S, I240L, N248A, D263G, G269D, R285D, D292G, H301T, K309R, K322G, R329A, L347F, L347Y, L347I, M351A, H353R, S361G, F362S, Y379S, L386A, K388G, H398A, K401R, K401D, T407S, L409G, S420T, P424A, F425S, P427A, D428G,  
15 H429A, Y430T, R431K, R431G, Y432S, Y432A, Y432F, and a combination thereof.

49. A conjugate, which conjugate comprises:

a) a mutant SAH hydrolase that has binding affinity for Hcy, SAH or adenosine but has attenuated catalytic activity, wherein said binding affinity and/or said attenuated catalytic  
20 activity of said SAH hydrolase is caused by a mutation in said mutant SAH hydrolase's catalytic site, its binding site for NAD<sup>+</sup>, NADH, Hcy, SAH or adenosine, or a combination thereof; and

b) a facilitating agent linked to the mutant SAH hydrolase directly or via a linker, wherein the agent facilitates:

- i) affinity isolation or purification of a conjugate;
- 25 ii) attachment of a conjugate to a surface; or
- iii) detection of a conjugate.

50. The conjugate of claim 49, which is a fusion protein.